

Full Length Research Paper

Antioxidant and *in-vitro* anthelmintic potentials of methanol extracts of barks and leaves of *Voacanga africana* and *Rauwolfia vomitoria*

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Voacanga africana (Stapf) and *Rauwolfia vomitoria* (Afzel) (Apocynaceae) are traditional plants widely used in folkloric medicine. Methanol extracts of *V. africana* bark (VAB) and leaves (VAL), and *R. vomitoria* bark (RVB) and leaves (RVL) were evaluated for antioxidant and anthelmintic potentials. The antioxidant properties of the extracts were determined by the DPPH free radical scavenging method using ascorbic acid as reference antioxidant. The IC₅₀ values were then determined. Four concentrations (20, 30, 40 and 50 mg/mL) of extracts were evaluated for *in-vitro* anthelmintic activity by determining the effects of the extracts on the paralytic and death time of *Pheretima posthuma* using albendazole (ABZ) (10 mg/mL) as reference standard. Results reveal that, all the extracts exhibited some level of antioxidant activity with IC₅₀ values of 187, 43, 610 and 967 µg/mL for VAL, RVB, VAB and RVL, respectively. VAB and RVB demonstrated significant anthelmintic activity. RVB at a concentration of 50 mg/mL had a paralytic time of 11.17 ± 0.088 min ($p < 0.001$) with reference to ABZ. It also demonstrated a concentration dependent reduction in death time of the worms at all concentrations tested. VAB demonstrated a concentration dependent effect on the worms with decreasing paralytic and death times upon an increase in extract concentration. It also showed significant paralytic and death times ($p < 0.001$) at concentrations of 30, 40 and 50 mg/mL with reference to albendazole.

Key words: Paralytic time, free radical, death time, anthelmintic activity, *Pheretima posthuma*, *Voacanga africana*, *Rauwolfia vomitoria*.

INTRODUCTION

Medicinal plants have been used for decades in the management of various ailments in ethno-medicine.

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Research has it that about 80% of the world's population especially in developing countries use plant materials as their source of primary health care (Farnsworth et al., 1985). Despite all the advancement in medical science, mankind still depends on medicinal plants as remedies to a number of ailments.

Anthelmintic resistance is now a serious problem particularly among farm animals and complete deworming of farm animals is currently very difficult (Prichard, 1994). High levels of drug resistance in human helminth infections such as soil-transmitted helminths (STH), (*Ascaris lumbricoides*, hookworms (*Necator americanus* and *Ancylostoma duodenale*) and *Trichuris trichiura*) have resulted from periodic mass administration of anthelmintic drugs to school age children and other at-risk groups (Vercruyssen et al., 2011). Thus in the face of drug resistance it is imperative that new molecules be sought to curb the menace.

Many medicinal plants have been used in the management of helminth infections and these include: *Carica papaya* (Levecke et al., 2014), *Annona senegalensis*, (Alawa et al., 2003) and essential oil of *Ocimum gratissimum* (Linn.) and eugenol, (Pessoa et al., 2002).

The seeds of *Voacanga africana*, (Stapf), are known to contain up to 10% indole alkaloids including voacamine and voacangine as well as many related compounds. Similar alkaloids are also found in the bark but in limited quantities (Bisset, 1985). Studies conducted have revealed that *V. africana* is a plant with a reservoir of alkaloids from which numerous alkaloid-based chemical compounds can be synthesized. Ibogaine, an alkaloid from *V. africana* has demonstrated numerous CNS effects (Kombian et al., 1997). *V. africana* has also been extensively studied for its alkaloids as well as its CNS and gastro-protective effects.

Rauwolfia vomitoria, (Afzel), (Apocynaceae) is a plant with numerous therapeutic uses (Irvine, 1961). Also known as serpent wood, (Kutalek and Prinz, 2007), the plant is traditionally used as treatment for snake bites, fever and nervous disorders (Kutalek and Prinz, 2007). The root, according to Prajapati et al. (2003) is a good anthelmintic and an antidote to snake venom. The root extracts are also known to possess good antioxidant effects (Okolie et al., 2011). Methanol extracts of the bark has demonstrated anti-ulcer activity in different models (Tan et al., 2000).

It is in this view that the leaves and barks of *V. africana* and *R. vomitoria* were evaluated to determine their effects on helminths and also explore their antioxidant potentials.

MATERIALS AND METHODS

Collection and preparation of plant material

Leaves and bark of *R. vomitoria* and *V. africana* were obtained from the forecourt of the Faculty of Pharmacy and Pharmaceutical Sciences, KNUST. They were authenticated by Dr. G. H. Sam, a Botanist in charge of the physique garden and herbarium of the

Department of Herbal Medicine, KNUST, where voucher specimens (KNUST/HM/2015L029 and KNUST/HM/2015L030 respectively) have been kept. The samples were thoroughly washed under running water to get rid of debris. The barks were sun dried for 5 days, while the leaves were dried at room temperature (25 to 28°C) for 5 days. The dried leaves and barks of *V. africana* and *R. vomitoria* were milled into powder using a laboratory mill machine (Type 8, Christy & Norris, UK). The powdered plant materials (50 g each) were each extracted by cold maceration for 72 h using methanol (70%v/v) and concentrated under reduced pressure using rotary vapour (Buchi, Germany). They were finally evaporated to dryness at 40°C in a hot-air oven and the weights of the extracts obtained recorded. The extracts were stored in a desiccator until needed. All chemicals used in the study were obtained from BDH, England, unless otherwise stated.

Experimental organism

Adult Indian earthworms (*Pheretima posthuma*; class Annelida; subclass Megascolecidae) which have anatomical and physiological resemblance to human intestinal roundworms (Vidarthi, 1967), were collected from the soil close to the Wiwi River in the Botanical Garden of KNUST. The earthworms were washed with 0.9% saline solution to remove all debris.

In-vitro anthelmintic bio-assay

An *in-vitro* anthelmintic bio-assay was performed according to the method described by Bhawar et al. (2009). *P. posthuma* samples, 4.0 to 5.0 cm in length and 0.10 to 0.20 cm in width were used. Extract solutions of concentrations of 20, 30, 40 and 50 mg/mL were prepared using a mixture of DMSO and distilled water in the ratio 2:8. Albendazole at a concentration of 10 mg/mL was used as the reference standard. A solution of 0.9% saline was used as the negative control.

Experimental procedure

The earthworms were placed in Petri dishes (five worms per Petri dish) into which the various extract solutions and reference standard were added. Observations were made for the times taken for the various extracts to cause paralysis and death of the individual worms. Paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Death was noted when the worms lost motility followed by a fading away of their body colour.

Determination of antioxidant activity

The antioxidant activity of the extracts were determined according to the method described in a previous study by Agyare et al. (2015) using the free radical 1,1-diphenyl-2-picryl-hydrazyl (DPPH) (Sigma- Aldrich, Damstadt, Germany). Solutions of the extracts and standard antioxidant (ascorbic acid) (Sigma-Aldrich, Damstadt, Germany) of concentrations 1.0, 3.0, 10.0, 30.0, 100.0, 300.0 and 1000.0 µg/mL were prepared in methanol. DPPH solution of concentration 5.0×10^{-6} M was prepared in methanol in a dark room. Three millilitres of this solution was added to 1.0 mL of the methanol test extracts and standard antioxidant. The tubes were kept in the dark for 30 min after which absorbance (A_1) of excess DPPH in the extracts and standard solutions were measured at a wavelength of 517 nm using a UV spectrophotometer. The absorbance (A_0) reading for a blank solution containing equivalent volumes of methanol and DPPH was used as control. The

Table 1. Paralysis time of RVB and RVL extracts against *P. posthuma*.

Extract conc. (mg/mL)	Time (min)		
	RVB	RVL	0.9% saline
20	19.38 ± 0.409	31.08 ± 0.260	Na
30	16.50 ± 0.500	26.64 ± 0.049	Na
40	15.68 ± 0.266	24.02 ± 0.044	Na
50	11.17 ± 0.088***	21.68 ± 0.095	Na
Paralysis time ABZ 10 mg/mL	15.48 ± 0.180		

RVB, *R. vomitoria* bark; RVL, *R. vomitoria* leaves; ABZ, Albendazole; Na, No activity; conc., concentration; *** $p < 0.001$.

Table 2. Paralysis time of VAB and VAL extracts against *P. posthuma*.

Extract conc. (mg/mL)	Time (min)		
	VAB	VAL	0.9% saline
20	16.62 ± 0.347	34.01 ± 0.720	Na
30	12.51 ± 0.289***	31.46 ± 0.395	Na
40	9.43 ± 0.536***	27.62 ± 0.968	Na
50	7.03 ± 0.491***	22.55 ± 0.569	Na
Paralysis time of ABZ (10 mg/mL)	15.48 ± 0.180		

VAB, *V. africana* bark; VAL, *V. africana* leaves; ABZ, Albendazole; Na, No activity; conc., concentration; *** $p < 0.001$.

Table 3. Death time of VAB and leaves extracts against *P. posthuma*.

Extract conc. (mg/mL)	Time (min)		
	VAB	VAL	0.9% saline
20	24.59 ± 0.356	141.71 ± 0.918	Na
30	19.07 ± 0.261	138.72 ± 1.254	Na
40	16.43 ± 0.780***	125.65 ± 0.872	Na
50	14.77 ± 0.117***	113.99 ± 1.014	Na
Death time ABZ (10 mg/mL)	21.03 ± 0.258		

VAB, *V. africana* bark; VAL, *V. africana* leaves; ABZ, Albendazole; Na, No activity; conc., concentration; *** $p < 0.001$.

percentage of free radical scavenged was calculated from the equation [% scavenging = $((A_0 - A_t) / A_0 \times 100)$]. The IC_{50} was determined as the concentration of samples which scavenged 50% of free DPPH radicals. The experiment was performed in replicates.

Statistical analysis

All results were plotted and analysed with GraphPad Prism 5.0 for windows (GraphPad software, San Diego, CA, USA) and analysed by two-way ANOVA followed by Bonferroni post-test analysis which recognises * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ as statistically significant.

RESULTS AND DISCUSSION

Anthelmintic activity

The extracts of *R. vomitoria* and *V. africana* demonstrated

a concentration dependent paralytic and death times on *P. posthuma* (Tables 1 to 4).

Antioxidant activity

VAL and RVB demonstrated relatively high antioxidant activity with reference to their IC_{50} as compared to ascorbic acid (Table 5 and Figure 1)

Studies conducted on the leaves and barks of *V. africana* and *R. vomitoria* revealed some pharmacological activity of the two plants. The methanol leaves and bark extracts of both plants demonstrated both anthelmintic and antioxidant activity. VAB demonstrated very potent anthelmintic activity with significant ($p < 0.001$) paralytic and death times with reference to albendazole. VAB demonstrated a concentration dependent activity with

Table 4. Death time of RVB and leaves extracts against *P. posthuma*.

Extract conc. (mg/mL)	Time (min)		
	RVB	RVL	0.9% saline
20	35.10 ± 0.608	185.68 ± 0.344	Na
30	27.02 ± 0.908	175.41 ± 0.455	Na
40	24.84 ± 0.182	167.10 ± 1.539	Na
50	21.67 ± 0.733	143.35 ± 1.413	Na
Death time ABZ 10 mg/mL	21.03 ± 0.258		

RVB, *R. vomitoria* bark;RVL, *R. vomitoria* leaves; ABZ, Albendazole; Na, No activity; conc., concentration.

Table 5. IC₅₀ of extracts and standard antioxidant.

Sample	IC ₅₀ µg/mL
VAL	187
VAB	610
RVL	967
RVB	43
ASCORBIC ACID	22

IC₅₀, concentration of agent that scavenged 50% of DPPH.

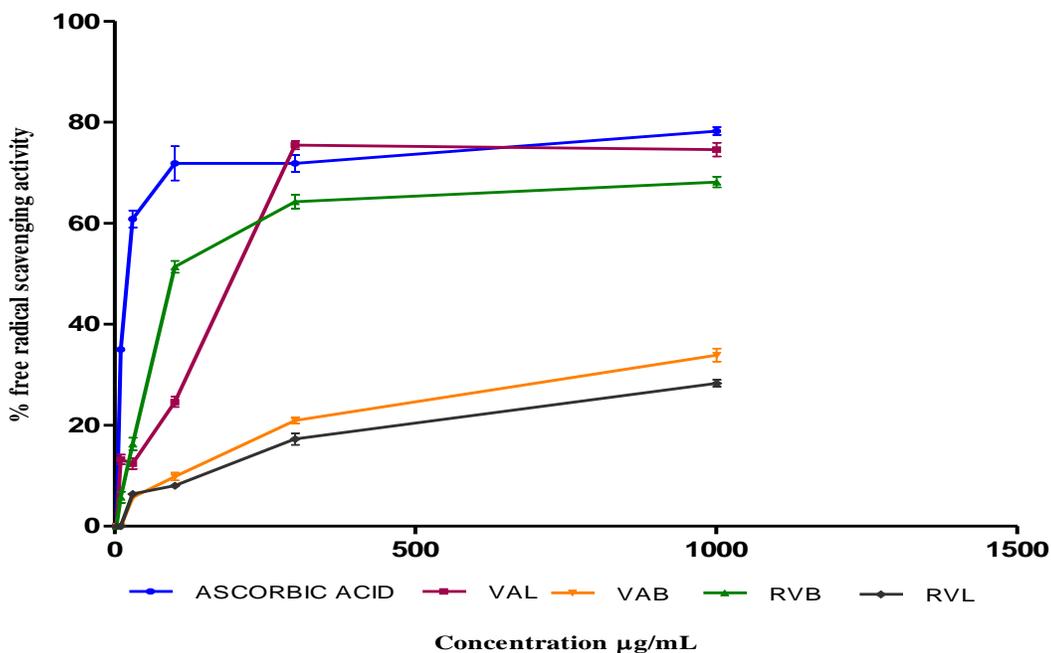


Figure 1. Antioxidant activity of methanol extracts of barks and leaves of *R. vomitoria*, *V. africana*, and ascorbic acid.

significant paralytic and death times ($p < 0.001$) at concentrations of 40 and 50 mg/mL (Tables 1 to 4). RVB

also demonstrated anthelmintic activity at all concentrations with a significant ($p < 0.001$) paralytic time at 50

mg/mL. The anthelmintic activity of plants have been attributed to the presence of some phytochemicals in the plants particularly tannins which are polyphenolic compounds (Olusegun-Joseph et al., 2012). Research has shown that some synthetic phenolic anthelmintics such as niclosamide, interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation or by binding to the free protein of the gastrointestinal tract of the worms. This eventually leads to death (Olusegun-Joseph et al., 2012). Tannins are presumed to exert the same effects on the worms. *V. africana* is known to possess tannins as some of its phyto-constituents (Ayoola et al., 2008). The anthelmintic effects demonstrated by the extracts could possibly be attributed to the presence of tannins in the extracts. Studies conducted on both plants have also revealed a wide array of alkaloidal content. The alkaloids can also cause paralysis of the worms by acting on its central nervous system (Mute, 2009), which could have also accounted for the anthelmintic effects of the extracts. The study revealed that the anthelmintic activities of the leaves of both plants are weaker (giving long paralysis and death times) than the barks that showed significant anthelmintic activities at concentrations of 30, 40 and 50 mg/mL ($p < 0.001$).

The study also revealed that the extracts possess good antioxidant activities. The IC_{50} values clearly depict the extent of antioxidant activity of the various extracts (Table 5). It was evident that the bark extracts of *R. vomitoria* demonstrated the highest free radical scavenging activity ($IC_{50} = 43 \mu\text{g/mL}$) with the lowest being RVL ($IC_{50} = 967 \mu\text{g/mL}$). The situation was however the opposite with *V. africana*, in which the leaves rather demonstrated much scavenging activity ($IC_{50} = 187 \mu\text{g/mL}$) than the bark ($IC_{50} = 610 \mu\text{g/mL}$). The antioxidant properties could be due to the presence of flavanoids and tannins which are known to exert antioxidant activity (Agyare et al., 2015; Marja et al., 1999). These two plants are already known to contain phytochemicals which include tannins and flavonoids (Okolie et al., 2011; Korochi et al., 2009) and might therefore account for the antioxidant activity. The two plants; *V. africana* and *R. vomitoria* could therefore, be potential sources of antioxidant compounds.

Conclusion

The methanol extracts of *R. vomitoria* and *V. africana* demonstrated anthelmintic activity with the bark extracts demonstrating significant anthelmintic activity. The extracts also demonstrated antioxidant activity at concentrations tested.

Conflict of interests

The authors did not declare any conflict of interest.

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